

CLAIMS.

1) A method for preparing nanoparticles coated with magnetic metal oxide, comprising the following steps:

a) Contacting an aqueous solution containing a soluble polymeric metal chelating agent with one or more soluble metal salts providing metal ions, wherein at least one of said metal ions is capable of forming an oxide which is magnetic, said metal ions being in amounts which do not exceed substantially the binding capacity of said chelating agent;

b) Causing said metal ions to be present in the oxidation states required for the formation of the oxide which is magnetic;

c) Maintaining the pH of the solution at the range of at least 7;

d) Introducing into the solution additional amounts of said metal salts;

e) Causing said additional metal ions to be present in the oxidation states required for the formation of the oxide which is magnetic;

f) Maintaining the pH of the solution at the range of at least 7;

g) Successively repeating the operations of step d) to f) as many times as required to obtain monodispersed nanoparticles coated with magnetic metal oxide.

2) A method according to claim 1, wherein the polymeric metal chelating agents have functional groups capable of binding metal ions selected from the group consisting of amino, hydroxyl, carboxylate, -SH, ether, immine, phosphate and sulfide groups.

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3) A method according to claim 1, wherein the polymeric metal chelating agent is selected from the group consisting of gelatin, polymethylenimine, dextran, chitosan, polylysine and polyvinylpyrrolidone.

4) A method according to claim 3, wherein the concentration of the polymeric metal chelating agent in the aqueous solution varies between 0.01 and 10 % w/v.

5) A method according to claim 4, wherein the concentration of the polymeric metal chelating agent in the aqueous solution varies between 0.1 to 1 % w/v.

6) A method according to claim 1, wherein the metal oxide which is magnetic is selected from the group consisting of iron oxides or ferrite.

7) A method according to claim 6, wherein the magnetic iron oxide is magnetite or maghemite, or a mixture thereof.

8) A method according to claim 1, wherein the aqueous solution is contacted with ferrous salts providing  $\text{Fe}^{+2}$  ions, and  $\text{Fe}^{+3}$  ions are caused to be present in the solution by oxidizing a portion of said  $\text{Fe}^{+2}$  ions.

9) A method according to claim 1, wherein the aqueous solution is contacted with a mixture of ferrous and ferric salts causing  $\text{Fe}^{+2}$  and  $\text{Fe}^{+3}$  ions to be present in the solution.

10) A method according to claim 8, wherein the oxidation of a portion of  $\text{Fe}^{+2}$  ions is carried out by introducing an oxidizer into the solution.

11) A method according to claims 6 and 10, wherein the magnetic metal oxide is iron oxide and the portion of  $\text{Fe}^{+2}$  which is oxidized is not higher than  $2/3$ , whereby the resulting molar ratio between  $\text{Fe}^{+2}$  to  $\text{Fe}^{+3}$  in the solution is not higher than 1:2.

12) A method according to claim 11, wherein the portion of  $\text{Fe}^{+2}$  which is oxidized is not higher than 1/2.

13) A method according to claim 10, wherein the oxidizer is selected from among oxygen,  $\text{H}_2\text{O}_2$ , nitrite or nitrate salts.

14) A method according to claim 13, wherein the oxidizer is  $\text{NO}_2^-$  or  $\text{NO}_3^-$ .

15) A method according to claim 12, wherein the molar ratio ( $\text{NO}_2^-$  or  $\text{NO}_3^-$ )/ $\text{Fe}^{+2}$  is not higher than 1/2.

16) A method according to claim 6, wherein the magnetic metal oxide is ferrite, further comprising adding in steps a) and d) transition metal salts.

17) A method according to claim 1, wherein the pH is maintained in the range of at least 7 by the addition of a base.

18) A method according to claim 1, wherein the pH is maintained at a constant value in the range between 8 to 10.

19) A method according to claim 1, wherein steps d) to f) are carried out in a portionwise mode of operation as hereinbefore defined.

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20) A method according to claim 1, wherein steps d) to f) are carried out in a continuous mode of operation as hereinbefore defined.

21) A method according to claim 1, wherein the size of the nanoparticles is less than  $0.1\mu\text{m}$

22) A method according to claim 1, wherein the temperature is between  $50^{\circ}\text{C}$  to  $90^{\circ}\text{C}$ .

23) A method according to claim 1, further comprising the removal of the inner polymeric metal chelating agent material to produce magnetic nanoparticles which are hollow, by burning off said polymeric material in inert atmosphere.

24) A method according to of claim 1 or 23, further comprising attaching to the magnetic surface of the magnetic nanoparticles molecules containing functional groups to produce desired functional coating on the particles.

25) A method according to claim 24 wherein the molecules containing functional groups comprise polymers selected from the group consisting of polysaccharides, proteins, peptides, polyamines and  $\omega$ -silane  $\text{Si}(\text{OR})_3(\text{CH}_2)_n\text{X}$ , wherein R is an alkyl substituent, n is an integer between 1 to 18, inclusive, and X is a functional group selected from the group consisting of  $\text{NH}_2$ ,  $\text{CH}_3$ ,  $\text{CN}$ , and  $\text{SH}$ .

26) A method according to claim 25, further comprising binding polyaldehyde ligands to the amine groups of the functional coating.

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27) A method according to claims 25 and ~~26~~ further comprising attaching activating ligands to the functional groups capable of binding bioactive agents.

28) A method according to claim 27 wherein the activating ligands are selected from the group consisting of acryloyl chloride, divinyl sulfone, dicarbonyl imidazole, ethylene glycolbis(sulfosuccinimidylsuccinate) and m-maleimidobenzoic acid N-hydroxysulfosuccinimide ester.

29) A method according to claim 28, further comprising coupling bioactive agents to the activating ligands.

30) A method according to claim 19 wherein the bioactive agents are compounds selected from the group consisting of proteins, enzymes, antibodies and drugs.

31) A method according to claim 1 for the microencapsulation of active materials within the magnetic nanoparticles, characterized in that an active material is introduced into the aqueous solution according to step a).

32) A method according to claim 31, wherein the active material is a drug or fluorescent dye.

33) A nanoparticle the size of which is less than  $0.3\mu\text{m}$ , consisting of a polymer which is metal chelating agent, coated with a magnetic metal oxide.

34) A nanoparticle according to claim 33, wherein its size is less than  $0.1\mu\text{m}$ .

35) A nanoparticle according to claim 34, wherein its size is less than 92 nm.

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36) A hollow nanoparticle consisting of a magnetic metal oxide shell the size of which is less than 0.3 $\mu$ m.

37) A hollow nanoparticle according to claim 36, wherein its size is less than 0.1 $\mu$ m.

38) A magnetic nanoparticle according to any of claims 34 to 37, further comprising a coating of a functional polymer on the magnetic coating.

39) A magnetic nanoparticle according to claim 38 wherein the functional polymeric coating comprises polymers selected from the group consisting of polysaccharides, proteins, peptides, polyamines and  $\omega$ -silane compounds.

40) A magnetic nanoparticle according to claim 39, bonded with activating ligands.

41) A magnetic nanoparticle according to claim 40 wherein the activating ligands are provided by compounds selected from the group consisting of acryloyl chloride, divinyl sulfone, dicarbonyl imidazole, ethylene glycolbis(sulfosuccinimidylsuccinate) and m-maleimidobenzoic acid N-hydroxysulfosuccinimide ester.

42) A magnetic nanoparticle according to claim 41 coupled to bioactive agents.

43) A magnetic nanoparticle according to claim 42 wherein the bioactive agent is a compound selected from the group consisting of proteins, enzymes, antibodies and drugs.

44) Microencapsule comprising a magnetic nanoparticle according to claim 33, 34 or 35, wherein an active

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material is enclosed within the magnetic metal oxide coating.

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45) Use of the magnetic nanoparticle according to any of claims 33 to 43 for biological or medical applications.

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46) Use of a magnetic nanoparticle according to claim 45, wherein said biological and medical applications are selected from the group consisting of cell labeling, cell separation, controlled release, diagnostics, enzyme immobilization, protein purification, drug delivery, contrast agents for MRI and sono-imaging applications and chelation of heavy metal ions.